AD_____
(Leave blank)

Award Number: W81XWH-13-1-0043

TITLE: Defining the Role of Autophagy Kinase ULK1 Signaling in Therapeutic Response of Tuberous Sclerosis Complex to mTOR Inhibitors

PRINCIPAL INVESTIGATOR: Reuben J. Shaw, Ph.D.

CONTRACTING ORGANIZATION: The Salk Institute for Biological Studies, La Jolla, CA 92037-1002

REPORT DATE: April 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

DISTRIBUTION STATEMENT: (Check one)

[X] Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
April 2014	Annual	1 April 2013 – 31 March 2014

4. TITLE AND SUBTITLE
Defining the Role of Autophagy Kinase ULK1 Signaling in Therapeutic Response of Tuberous Sclerosis Complex to mTOR Inhibitors

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0043

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Shaw, Reuben J.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Salk Institute for Biological Studies
10010 North Torrey Pines Road
La Jolla, CA 92037-1002

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The Tuberous Sclerosis Complex tumor suppressors are known to be critical negative regulators of the mTORC1 kinase complex that controls cell growth and autophagy. Our laboratory and others have recently decoded a major conserved route that mTORC1 uses to control autophagy. These studies demonstrate that mTORC1 inactivates another kinase complex composed of the autophagy kinase ULK1 and its associated subunits. One prediction of these findings is that in cells and tumors with TSC mutations and hyperactive mTOR, the ULK1 complex – and the process of autophagy – will be suppressed. There were two major aims for this funding period: 1) to better define phosphorylation sites newly identified ULK1 substrates to be used as biomarkers of mTOR inhibition, and 2) to determine whether novel small molecule inhibitors of ULK1 convert the cytostatic effects of mTOR inhibitors into cytotoxic effects when used in combination.

15. SUBJECT TERMS
autophagy, cell survival, ULK1, phosphorylation, substrates, mTOR, rapamycin

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	14	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

INTRODUCTION

The Tuberous Sclerosis Complex tumor suppressors are known to be critical negative regulators of the mTORC1 kinase complex that controls cell growth and autophagy. Our laboratory and others have recently decoded a major conserved route that mTORC1 uses to control autophagy. These studies demonstrate that mTORC1 inactivates another kinase complex composed of the autophagy kinase ULK1 and its associated subunits. One prediction of these findings is that in cells and tumors with TSC mutations and hyperactive mTOR, the ULK1 complex – and the process of autophagy – will be suppressed. There were two major aims for this funding period: 1) to better define phosphorylation sites newly identified ULK1 substrates to be used as biomarkers of mTOR inhibition, and 2) to determine whether novel small molecule inhibitors of ULK1 convert the cytostatic effects of mTOR inhibitors into cytotoxic effects when used in combination.

BODY

We have made significant progress on Task 1 to examine how ULK1 activity and function are altered by mTOR inhibitors. First, to develop a robust ULK1 kinase assay, we identified the optimal ULK1 substrate motif in order to develop a peptide that could serve as an artificial substrate to functionally read-out ULK1 catalytic activity. We utilized spatially arrayed degenerate scanning peptide libraries to define the optimal substrate motif for ULK1 (see Figure 1). The motif identified bears striking sequence homology to the optimal sequence recently reported for Atg1, a homolog from budding yeast with a similar function[1]. From the optimal ULK1 substrate motif we defined, we are currently characterizing ULK1 IP-kinase activity towards a peptide substrate bearing the optimal sequence following different mTOR inhibitor treatments in cells.

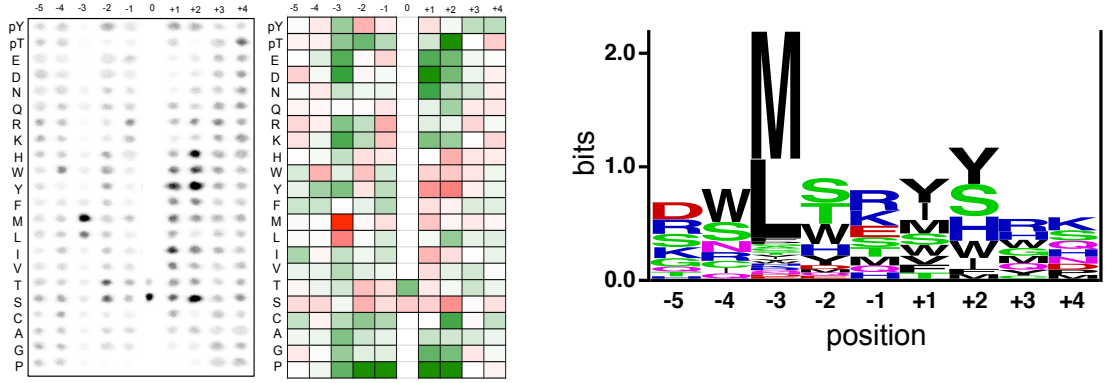

Figure 1. Degenerate arrayed peptide libraries were used to determine the optimal substrate motif for human ULK1. The selectivity of the kinase motif is illustrated graphically at the right, with the position of each residue relative to the phospho-acceptor serine listed.

Task 2 was aimed at further identifying ULK1 substrates in vivo whose induction of phosphorylation by ULK1 may serve as functional biomarkers for mTOR inhibition in cells. Here we have also made significant advances, identifying a number of new direct ULK1 substrates. As seen in Figure 2 for the ULK1-binding protein ATG101[2, 3], only two specific serines amidst the tens and tens of serines in the protein can serve as direct ULK1-phosphorylation sites. The sequence of these two sites also conforms to the optimal substrate motif identified above (see Figure 2).

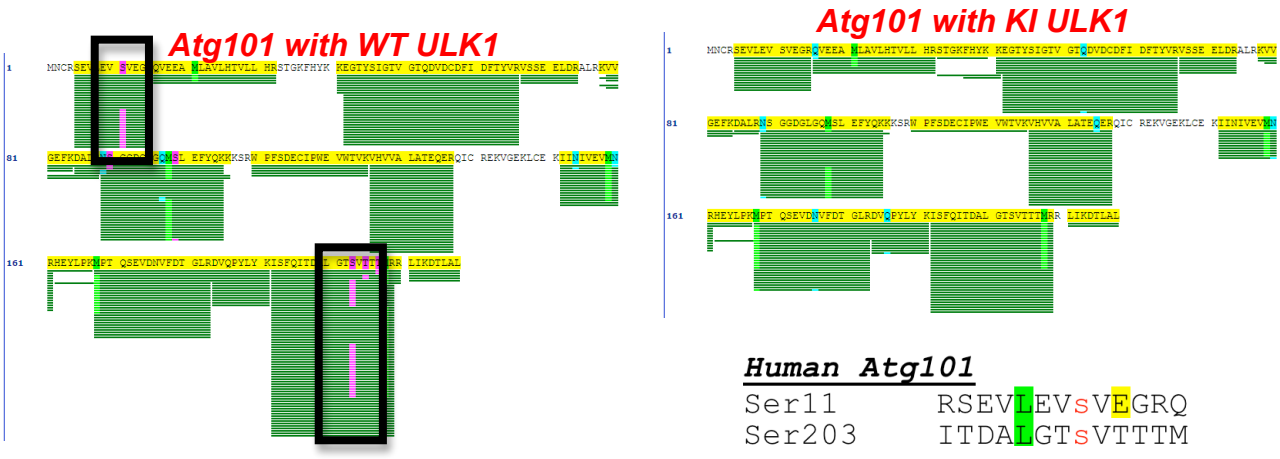

Figure 2. In vivo phosphorylation sites were defined in the ULK1-binding partner ATG101 when it was co-expressed with wild-type (WT) or kinase inactive (KI) ULK1 in HEK293T cells. We identified two specific serines in ATG101 which match the optimal ULK1 substrate consensus (LxxpSVxx).

Task 3 and 4 lie outside of what was anticipated for the initial year of funding, though the effects of ULK1 inhibitors on cell growth, as in Task 3, are also further explored in Task 5, which is a final Task that we have also made significant advances in. In Task 5, we propose to spend the first year of funding defining how well novel catalytic inhibitors of ULK1 we test and help validate can act as cytostatic or cytotoxic agents for treatment with nutrient starvation or treatment with mTOR catalytic inhibitors. We now have a tool compound, Compound 6, that serves this exact purpose and shows excellent catalytic inhibition towards ULK1 (see Figure 3). As currently there has not been a single reported ULK1 inhibitor in the literature, and ULK1 is the only druggable kinase in the classic autophagy pathway, we believe these results will be of great interest to many, and we are preparing to submit a paper in the next 2 months including all of the findings reported in this annual report.

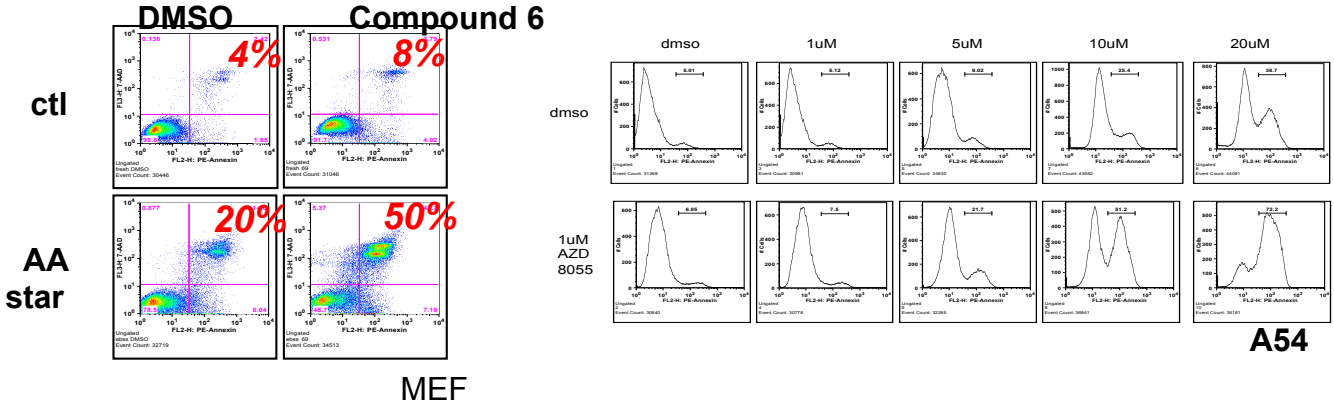

Figure 3. A novel ULK1 ATP-competitive inhibitor was identified and used to test whether ULK1 pharmacological inhibition mimics the effects seen with genetic loss of ULK1 by RNAi or genetic knockout[4]. As seen above left in MEFs combined with Amino Acid starvation, or above right in A549 tumor cells with the mTOR catalytic inhibitor AZD8055, the ULK1 inhibitor Compound 6 synergized to induce cell death as readout by AnnexinV staining.

KEY RESEARCH ACCOMPLISHMENTS

- identification of ULK1 phosphorylation sites in 4 protein to act as biomarkers in vivo
- identification of a ULK1 inhibitor tool compound that synergizes with mTOR inhibitors to trigger death of cancer cells

REPORTABLE OUTCOMES

Preparing manuscript to submit in the next few months describing multiple outcomes of the experiments mentioned here.

CONCLUSION

Our findings during this first year of funding have been very fruitful, accomplishing multiple Tasks as proposed in the Statement of Work of the grant. In particular, we have identified multiple ULK1 substrates in vivo and unambiguously identified the ULK1 phosphorylation sites, and have shown these sites are induced in cells treated with mTOR inhibitors as hypothesized originally. This suggests that the extent of phosphorylation of these sites in vivo may possibly be used as a biomarker for the extent of mTOR inhibition. Given the widespread use of mTOR inhibitors including rapalogs to treat Tuberous Sclerosis Complex, the identification of new biomarkers for TSC treatment is particularly exciting. In addition, we have pursued the identification of small molecule ATP-competitive inhibitors of ULK1 and now have a very interesting tool Compound 6 that synergizes converts the cytostatic growth arrest of cells starved of nutrients into a cytotoxic cell death response, consistent with our previous studies demonstrating that ULK1 provides cell survival signal when nutrients are deprived. We are now testing further how well this compound synergizes with mTOR catalytic inhibitor in cancer cell lines and ultimately mouse models, consistent with the aims of the grant proposal.

REFERENCES

1. Papinski, D. *et al.* Early steps in autophagy depend on direct phosphorylation of Atg9 by the Atg1 kinase. *Mol Cell* **53**, 471-483 (2014).
2. Hosokawa, N. *et al.* Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy* **5**, 973-979 (2009).
3. Mercer, C.A., Kaliappan, A. & Dennis, P.B. A novel, human Atg13 binding protein, Atg101, interacts with ULK1 and is essential for macroautophagy. *Autophagy* **5**, 649-662 (2009).
4. Egan, D.F. *et al.* Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* **331**, 456-461 (2011).

APPENDICES

Shaw CV.

Reuben James Shaw, Ph.D. Curriculum Vitae 4/15/14

General Information

Reuben J. Shaw
Professor
Howard Hughes Medical Institute
Molecular and Cell Biology Laboratory (MCBL-W)
The Salk Institute for Biological Studies
10010 North Torrey Pines Road
La Jolla, CA 92037
Tel: 858-453-4100 x1258
Email: shaw@salk.edu

Education

09/89-06/93 B.S., Biology, Cornell University, Ithaca, NY graduated with highest honors (summa cum laude)
09/93-06/99 Ph.D., Biology, Massachusetts Institute of Technology, Cambridge, MA

Research Experience/ Training

1991-1993	Undergraduate research, Cornell University	Advisor: Dr. Richard Cerione, Ph.D.
1994-1999	Ph.D., Massachusetts Institute of Technology	Advisor: Dr. Tyler Jacks, Ph.D.
1999-2003	Postdoctoral Fellow, Harvard Medical School	Advisor: Dr. Lewis Cantley, Ph.D.
2003-2005	Instructor, Harvard Medical School	Advisor: Dr. Lewis Cantley, Ph.D.
2006-2012	Assistant Professor, Salk Institute	Molecular & Cell Biology Laboratory
2012-2014	Associate Professor, Salk Institute	Molecular & Cell Biology Laboratory
2014-present	Professor, Salk Institute	Molecular & Cell Biology Laboratory

Honors and Awards

2006-2009 The V Foundation for Cancer Research Scholar Award
2007-2011 American Cancer Society Research Scholar
2008-2011 American Diabetes Association Junior Faculty Award
2009-2015 Howard Hughes Medical Institute Early Career Scientist Award

Additional Institutional Affiliations

2006-present Assistant Adjunct Professor, Division of Biological Sciences, UCSD
2009-present Assistant Adjunct Professor, Department of Medicine, UCSD

Institutional Service

2008-present Salk Academic Council
2008-2011 Administered postdoctoral fellow training grant in Salk Helmsley Nutritional Genomics program
2011-present Salk Faculty Development Committee
2009-present Created and ran Salk Cancer Course for T32 NCI Cancer Center training grant; developed into a cross-listed upperclassman / masters Ph.D. student UCSD course BGGN245
2006- present Teach UCSD undergrads and grad courses ~ 5 lectures / year
2006- present UCSD-Salk graduate student recruitment, thesis committees, training grant workshops
2013- present Associate Program Leader, Salk Cancer Center

Extramural Professional Responsibilities

2010-present Editorial Board, Oncogene
2010-present Editorial Board, Biochemical Journal
2007-present Manuscript Reviewer: Nature, Cell, Science, Nature Medicine, Nature Cell Biology, Molecular Cell, Cell Metabolism, Cancer Cell, JCI, Science Signaling, MCB, PNAS, PLOS, JBC, G&D, Cancer Research, Biochemical Journal, and Oncogene
2009-present Grant Reviewer: NIH (NCI, NIDDK), American Cancer Society, Tuberous Sclerosis Alliance
2009-2012 Member, Grant Review Board, State of Texas Cancer Research Initiative (CPRIT)

Of great importance to the field, I have served as a co-organizer for a number of international meetings:

Meeting Co-organizer

2009 AACR Meeting on Cancer & Metabolism (w/ Ron Evans & Celeste Simon)
2009 Salk Cancer Mechanisms & Models (w/ Jan Karlseder, Laura Attardi, Clodagh Oshea & Dave Tuveson)
2011 Salk Cancer Mechanisms & Models (w/ Jan Karlseder, Laura Attardi, Clodagh Oshea & Dave Tuveson)
2012 Keystone Meeting on Cancer Metabolism (w/ David Sabatini)
2013 FASEB Meeting on Protein Kinase and Protein Phosphorylation (w/ Richard Marais)
2013 Salk Cancer Mechanisms & Models (w/ Jan Karlseder, Laura Attardi, Clodagh Oshea & Dave Tuveson)

Invited Talks given

2013 Other invited talks given beyond meetings I co-organized: 25th Pezcoller Symposium Trento Italy Banbury Meeting on Biguanides, CSHL meeting on Metabolism & Disease, Yale University Physiology Dept, Memorial Sloan-Kettering Cancer Center, Oregon Health Science University, Keystone Tumor Metabolism Meeting, Annual AACR meeting, World Congress on Insulin Resistance, Diabetes & Cardiovascular Disease

2012 Other invited talks given beyond meetings I co-organized: Banbury Meeting on Cancer Metabolism, Les Treilles TOR Meeting, St. Jude's 50th Anniversary Symposium, annual Forbeck Symposium, FASEB Meeting on AMPK, EPFL School of Life Sciences, London Research Institute, IPSEN Cancer Meeting on Mouse Models, Albert Einstein Cell Biology Dept, Dartmouth Medical School, annual American Diabetes Association Meeting, annual Endocrine Society Meeting

2011 Other invited talks given beyond meetings I co-organized: CSHL 76th Annual Symposium on Metabolism & Disease, Molecular and Physiological Aspects of Type 2 Diabetes and Obesity Symposium at the Karolinska Institute, Keystone Diabetes Meeting, Aspen Cancer Conference, FASEB Meeting on Kinases and Protein Phosphorylation, FASEB Meeting on Nutrient Control and Metabolism, AACR Meeting on Metabolism and Cancer, Banbury Meeting on Metformin and Cancer, University of Utah Metabolism Symposium, Washington University Cell Biology Dept, University of Pennsylvania Cancer Center, Stanford Cancer Center, McGill Cancer Center, Massachusetts General Hospital Cancer Center, UTSW Children's Hospital Cancer Institute

2010 Other invited talks given where also served as session chair: American Association of Cancer Research (AACR) Annual Meeting, Metabolism in Cancer Conference, Berlin, Germany, Gordon Conference on Cell Growth and Proliferation, The tumor suppressor LKB1: basic science to the clinic in Marseilles France, FASEB 5th International AMPK Conference in Kyoto

2010 Other invited talks given at meeting or weekly seminar series: Keystone Meeting on Cancer & Metabolism, CSHL PTEN meeting, Fred Hutchinson Cancer Center, Dana Farber Cancer Center, CSHL 5th Annual Cancer Mechanisms & Models, Abcam Cancer and Metabolism Meeting, Banbury Meeting on Cancer Metabolism, Barcelona Biomed Conference on Cancer Metabolism, Vanderbilt Weekly Physiology Seminar Series, NCI Workshop on Cancer and Autophagy

2009 Other invited talks given at meeting or weekly seminar series: CNIO Meeting on Cancer and Metabolism, American Cancer Society Professors Meeting, Abcam Meeting on Aging and Age-related disease, FASEB Meeting on Kinases and Protein Phosphorylation, Duke University Department of Pharmacology, Case Western University Dept of Pathology, Forbeck Foundation Annual Meeting, Tuberous Sclerosis Alliance International Symposium, UCLA IMED Seminar Series, Yale Medical School Pharmacology Weekly Seminar, Memorial Sloan-Kettering Cancer Center Cancer Biology weekly Seminar

2008 Other invited talks: American Association of Cancer Research (AACR) Annual Meeting, CNIO Cancer Conference on mTOR Signaling Metabolism & Cancer, CSHL 3rd Annual Cancer Mechanisms & Models, FASEB Meeting on Nutrient Regulation, FASEB International AMPK Meeting in Copenhagen, IPSEN Cancer and Metabolism Meeting, Minisymposium at Fred Hutchinson Cancer Research Center, Beatson Institute Cancer & Metabolism Symposium, UNC

Lineberger Comprehensive Cancer Center Weekly Seminar, Boston Medical Center Whittaker Cardiovascular Weekly Seminar, Annual LAM (Lymphoangiomyoleiosarcoma) Foundation Meeting, UC Irvine Molecular and Cell Biology Weekly Seminar, UT Southwestern Pathology Weekly Seminar, UT Southwestern Pharmacology Weekly Seminar, UPenn Mari Lowe Center for Comparative Oncology Weekly Seminar, National Cancer Institute Distinguished Scientist Lecture

2007 Other invited talks: Gordon Conference on Signaling in the Nucleus, Keystone Symposium on Nuclear Receptor Pathways and Metabolic Syndrome, American Association of Cancer Research Annual Meeting, American Diabetes Association Annual Meeting, Gordon Conference on Cell Growth & Proliferation, FASEB Meeting on Protein Kinases, Kern Lipid Conference, UCLA Hillblom Diabetes Symposium on Tumor Suppressors and Diabetes, UC Davis Cancer Center Weekly Seminar, UCSD Mahajani Symposium on Cancer & Metabolism, International Tuberous Sclerosis Alliance Annual Meeting, University of Wisconsin at Madison Weekly Seminar

2006 Keystone Diabetes Meeting, Nutrient Sensing, NCI Insulin Signaling and Hamartoma Syndromes Meeting, UCSF Cancer Center, Gordon Conference on Phosphorylation & G Protein Mediated Signaling, FASEB Meeting on AMPK, Annual Obesity Society Conference

Shaw Lab Personnel as of April 30, 2014

10 postdoctoral fellows, 3 PhD students, 3 research technicians

Former Trainees

1st postdoc David Shackelford finished 2011; now an Assistant Professor at UCLA Medical School
Will Mair, a joint postdoc with Andrew Dillin finished 2011; now Asst Prof at Harvard School of Public Health
Jung-Whan (Jay) Kim, joint postdoc with Randy Johnson finished 2013, now Asst Prof at UT Dallas
1st PhD student to graduate Dana Gwinn is now a postdoc at Stanford
2nd PhD student to graduate Maria Mihaylova is now a postdoc at the Whitehead Institute at MIT
3rd PhD student to graduate Rebecca Kohnz is now a postdoc at UC Berkeley.
4th PhD student to graduate Daniel Egan is now a postdoc at Harvard Medical School

Publications – Primary Research

1. Luan, B., Goodarzi, M.O., Phillips, N.G., Guo, X., Chen, Y.I., Yao, J., Allison, M., Rotter, J.I., **Shaw, R.J**., and Montminy, M. (2014) Leptin-mediated increases in Catecholamine signaling reduce adipose tissue inflammation via activation of Macrophage HDAC4. ***Cell Metab*** (in press).

2. Faubert, B., Vincent, E.E., Griss, T., Samborska, B., Izreig, S., Svensson, R.U., Mamer, O.A., Avizonis, D., Shackelford, D.B., **Shaw, R.J.**, and Jones, R.G. (2014) Loss of the tumor suppressor LKB1 promotes metabolic reprogramming of cancer cells via HIF-1a. ***Proc Natl Acad Sci USA*** 111: 2554-2559.

3. Masui, K., Tanaka, K., Akhavan, D., Babic, I., Gini, B., Matsutani, T., Iwanami, A., Liu, F., Villa, G.R., Gu, Y., Campos, C., Zhu, S., Yang, H., Yong, W.H., Cloughesy, T.F., Mellinghoff, I.K., Cavenee, W.K., **Shaw, R.J**., and Mischel, P.S. (2013) mTOR Complex 2 Controls Glycolytic Metabolism in Glioblastoma through FoxO Acetylation and Upregulation of c-Myc. ***Cell Metab*** 18: 726-739.

4. Liu, Y., Marks, K., Cowley, G.S., Carretero, J., Liu, Q., Nieland, T.J., Xu, C., Cohoon, T.J., Gao, P., Zhang, Y., Chen, Z., Altabef, A.B., Tchaicha, J.H., Wang, X., Choe, S., Driggers, E.M., Zhang, J., Bailey, S.T., Sharpless, N.E., Hayes, D.N., Patel, N.M., Janne, P.A., Bardeesy, N., Engelman, J.A., Manning, B.D., **Shaw, R.J.,** Asara, J.M., Scully, R., Kimmelman, A., Byers, L.A., Gibbons, D.L., Wistuba, I.I., Heymach, J.V., Kwiatkowski, D.J., Kim, W.Y., Kung, A.L., Gray, N.S., Root, D.E., Cantley, L.C., and Wong, K.K. (2013) Metabolic and functional genomics identify deoxythymidylate kinase as a target in LKB1-mutant lung cancer. ***Cancer Discov*** 3: 870-9.

5. Shackelford, D.B, Abt, E., Gerken, L., Vasquez, D.S., Seki, A., Leblanc, M., Wei, L., Fishbein, M.C., Czernin, J., Mischel, P.S., and **Shaw, R.J**. (2013) LKB1 inactivation dictates therapeutic response of non-small cell lung cancer to the metabolism drug phenformin. ***Cancer Cell*** 23: 143-158.

6. Xia, Y., Yeddula, N., LeBlanc, M., Ke, E., Zhang, Y., Oldfield, E., **Shaw, R.J**., and Verma, I.M. (2012) Reduced cell proliferation by IKK2 depletion in a mouse lung-cancer model. ***Nat Cell Biol*** 14: 257-65.

7. Mihaylova, M.M., Vasquez, D.S., Ravnskjaer, K., Denechaud, P.D., Yu. R.T., Alvarez, J.G., Downes, M., Evans. R.M., Montminy, M., and **Shaw, R.J.** (2011) Class IIa histone deacetylases are hormone-activated regulators of FOXO and mammalian glucose homeostasis. ***Cell***,145: 607-621.

8. Li, Y., Xu, S., Mihaylova, M., Zheng, B., Hou, X., Jiang, B., Park, O.,Luo, Z., Lefai, E., Shyy, J.Y., Gao, B., Wierzbicki, M., Verbeuren, T.J., **Shaw, R.J.**, Cohen, R.A., and Zang, M. (2011) AMPK Phosphorylates and Inhibits SREBP Activity to Attenuate Hepatic Steatosis and Atherosclerosis in Diet-induced Insulin Resistant Mice. ***Cell Metabolism*** 13: 376-88.

7. Mair, W., Morantte, I., Rodrigues, A.P., Manning, G., Montminy, M., **Shaw, R.J.*** and Dillin, A*. (2011) Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. ***Nature*** 470: 404-8. (*co-corresponding authors)

8. Egan, D.F., Shackelford, D.B., Mihaylova, M.M., Gelino, S.R., Kohnz, R.A., Mair, W., Vasquez, D.S., Joshi, A., Gwinn, D.M., Taylor, R., Asara, J.M., Fitzpatrick, J., Dillin, A., Viollet, B., Kundu. M., Hansen, M. and **Shaw, R.J.** (2010) Phosphorylation of ULK1 (hATG1) by AMP-Activated Protein Kinase Connects Energy Sensing to Mitophagy. ***Science*** 331: 456-61.

9. Gwinn, D.M., Asara, J. and **Shaw, R.J.** (2010) Raptor is phosphorylated by cdc2 during mitosis. ***PLoS ONE*** 5: e9197.

10. Ohashi, K., Ouchi, N., Higuchi, A., **Shaw, R.J.,** and Walsh**,** K. (2010) LKB1 deficiency in Tie2-Cre expressing cells impairs ischemia-induced angiogenesis. ***J Biol Chem*** 285: 22291-8.

11. Ikeda, Y., Sato, K., Pimentel, D.R., Sam, F., **Shaw, R.J**., Dyck, J.R., Walsh, K. (2009). Cardiac-specific deletion of LKB1 leads to hypertrophy and dysfunction. ***J Biol Chem*** 284: 35839-49.

12. Lamia, K.A., Sachdeva, U.M., DiTacchio, L., Williams, E.C., Alvarez, J.G, Egan, D.F., Vasquez, D.S., Juguilon, H., Panda, S., **Shaw, R.J.**, Thompson, C.B., and Evans, R.M. (2009) AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. ***Science*** 326: 437-40.

13. Shackelford, D.B., Vasquez, D.S., Corbeil, J., Wu, S., Leblanc, M., Wu, C.-L., Vera, D.R. and **Shaw, R.J**. (2009) mTOR and HIF-1α mediated tumor metabolism in an LKB1 mouse model of Peutz-Jeghers syndrome. ***Proc Natl Acad Sci USA*** 106: 11137-42.

14. Mair, W., Panowski, S.H., **Shaw, R.J.**, and Dillin, A. (2009) Optimizing Dietary Restriction for Genetic Epistasis Analysis and Gene Discovery in C. elegans. ***PLOSOne***, 4: e4535.

15. Narkar, V.A., Downes, M., Yu, R.T., Embler, E., Wang, Y.X., Banayo, E., Mihaylova, M.M., Nelson, M.C., Zou, Y., Juguilon, H., Kang, H., **Shaw, R.J.** and Evans, R.M. (2008) AMPK and PPARdelta agonists are exercise mimetics. ***Cell*** 134: 405-15.

16. Gwinn, D.M., Shackelford, D.B., Egan., D.F., Mihaylova, M.M., Mery, A., Vasquez, D.S., Turk, B.E. and **Shaw, R.J.** (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. ***Mol Cell*** 30: 214-26.

17. Baur, J.A., Pearson, K.J., Price, N.L., Jamieson, H.A., Lerin, C., Kalra, A., Prabhu, V.V., Allard, J.S., Lopez-Lluch, G., Lewis, K., Pistell, P.J., Poosala, S.Becker, K.G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K.W., Spencer, R.G., Lakatta, E.G., LeCouteur, D., **Shaw, R.J.**, Navas, P., Puigserver, P., Igram, D.K., deCabo, R., Sinclair, D.A. (2006) Resveratrol improves health and survival of mice on a high-calorie diet. ***Nature*** 444: 337-42.

18. **Shaw, R.J**.*, Lamia, K.A., Vasquez, D., Koo, S.H., Bardeesy, N., DePinho, R.A., Montminy, M., Cantley, L.C. (2005) The Kinase LKB1 Mediates Glucose Homeostasis in Liver and Therapeutic Effects of Metformin. ***Science*** 310: 1642-6. (*corresponding author)

19. Fernandes, N., Sun, Y., Chen, S., Paul, P., **Shaw, R.J**., Cantley, L.C., Price, B.D. (2005) DNA damage-induced association of ATM with its target proteins requires protein interaction domain in the N terminus of ATM. ***J Biol Chem*** 280: 15158-64.

20. **Shaw, R.J**., Bardeesy, N., Manning, B., Lopez, L. Kosmatka, M., DePinho, R.A., and Cantley, L.C. (2004) The LKB1 tumor suppressor negatively regulates mTOR signaling. ***Cancer Cell*** 6: 91-99.

21. **Shaw, R.J**., Kosmatka, M., Bardeesy, N., Hurley, R.L., Witters, L.A., DePinho, R.A., Cantley, L.C. (2004) The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. ***Proc Natl Acad Sci USA*** 101: 3329-35.

22. Karuman, P., Gozani, O., Odze, R., Zhou, X.C., Zhu, H., **Shaw, R.**, Brien, T.P., Bozzuto, C.D., Ooi, D., Cantley, L.C., and Yuan, J. (2001) The Peutz-Jegher Gene Product LKB1 is a mediator of p53-dependent cell death. ***Molecular Cell*** 7: 1307-19.

23. **Shaw, R.J.**, Paez, J.G., Curto, M., Yaktine, A., Pruitt, W.M., Saotome, I., O'Bryan, J.P., Gupta, V., Ratner, N., Der, C.J., Jacks, T. and McClatchey, A.I. (2001) The Nf2 tumor suppressor, merlin, functions in Rac-dependent signaling. ***Developmental Cell*** 1: 63-72.

24. **Shaw, R.J.**, McClatchey, A.I., and Jacks, T. (1998) Regulation of the Neurofibromatosis type 2 tumor suppressor protein, merlin, by adhesion and growth arrest stimuli. ***J Biol Chem*** 273: 7757-64.

25. **Shaw, R.J**., McClatchey, A.I., and Jacks, T. (1998) Localization and functional domains of the Neurofibromatosis type II tumor suppressor, merlin. ***Cell Growth Diff*** 9: 287-96.

26. Platko, J., Leonard, D., Adra, C., **Shaw, R.J**., and Cerione, R.A. (1995) A single residue can modify target binding affinity and activity of the functional domain of the Rho-subfamily GDP dissociation inhibitors. ***Proc Natl Acad Sci USA*** 92: 2974-8.

Publications – Reviews, Essays, Previews, etc

1. Mouchiroud, L., Eichner, L.J., **Shaw, R.J**, and Auwerx, J. (2014) Transcriptional coregulators – fine-tuning metabolism. ***Cell Metabolism*** (*in press*)

2. **Shaw, R.J.** (2013) Metformin trims fats to restore insulin sensitivity. ***Nat Med*** 19, 1570-2.

3. **Shaw, R.J.** (2013) GATORs take a bite out of mTOR. ***Science*** 340: 1056-7.

4. Chun, M.G. and **Shaw, R.J.** (2013) Cancer metabolism in breadth and depth. ***Nat Biotech***. 31:505-7.

5. Mihaylova, M.M. and **Shaw, R.J**. (2013) Metabolic Reprogramming by Class I and II histone deacetylases. ***Trends Endo Metab*** 24: 48-60.

6. Svensson, R.U. and **Shaw, R.J.** (2012) Cancer metabolism: tumor friend or foe. ***Nature*** 485: 590-1.

7. **Shaw, R.J.** and Cantley, L.C. (2012) Cell biology: ancient sensor for ancient drug. ***Science*** 336: 813-4.

8. **Shaw, R.J.** and Cantley, L.C. (2012) Decoding key nodes in the metabolism of cancer cells: sugar & spice and all things nice. ***F1000 Reports*** 4: 2 (doi:10.3410/B4-2).

9. Mihaylova, M.M. and **Shaw, R.J.** (2011) The AMPK pathway coordinates cell growth, autophagy, and metabolism. ***Nature Cell Biology*** 13: 1016-23.

10. Akhtar, A., Fuchs, E., Mitchison, T., **Shaw, R.J.**, St. Johnston, D., Strasser, A., Taylor, S., Walczak, C., Zerial, M. (2011) A decade of molecular cell biology: achievements and challenges. ***Nat Rev Mol Cell Biol*** 12: 669-774.

11. Birnbaum, M.J. and **Shaw, R.J.** (2011) Genomics: Drugs, diabetes, and cancer. ***Nature*** 470: 338-9.

12. Gwinn, D.M. and **Shaw, R.J**. (2010) AMPK control of mTOR signaling and growth. ***The Enzymes*** 28: 49-75.

13. Shaw, R.J., Evans, R.M., and Simon, M.C. (2010) Metabolism and cancer in La Jolla. ***Cancer Research*** 70: 3864-9.

14. Shaw, R.J., (2010) Metabolism and cancer mix in Madrid. ***EMBO Rep*** 11: 249-251.

15. Shackelford, D.B. and Shaw, R.J. (2009) The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. ***Nat. Rev. Cancer*** 9: 563-75.

16. Mihaylova, M.M. and Shaw, R.J. (2009) The LKB1-AMPK pathway controls hepatic metabolism and therapeutic response. ***The Liver***, 5th edition, Wiley-Blackwell, pp.535-49.

17. Shaw, R.J. (2009) LKB1 and AMP-activated protein kinase control of mTOR signalling and growth. ***Acta Physiol*** 196: 65-80.

18. Shaw, R.J**.** (2009) Tumor suppression by LKB1: SIK-ness prevents metastasis. ***Sci Signaling*** 1: pe65

19. Shaw, R. and Dillin, A. (2009) PPTR-1 counterAkts insulin signaling. ***Cell*** 136: 816-18.

20. Shaw, R.J. (2008) Raptor swoops in on metabolism. ***Cell Metabolism*** 8: 343-4.

21. Shaw, R.J. (2008) LKB1: cancer, polarity, metabolism, and now fertility. ***Biochemical Journal*** 416: e1-3.

22. Shaw, R.J. (2008) mTOR signaling: RAG GTPases transmit the amino acid signal. ***Trends Biochem Sci,*** 33: 565-8.

23. Shaw, R.J. (2006) Glucose metabolism and cancer. ***Curr Opin Cell Biol*** 18*:* 598-608.

24. Shaw, R.J. and Cantley, L.C. (2006) Ras, PI3(K), and mTOR signaling control tumor cell growth. ***Nature*** 441: 424-30.

Research Support

ACTIVE

5 R01 CA172229-02 (Shaw, PI) 01/16/2013-12/31/2017
NIH/NCI
AMPK and AMPK-related kinases in lung cancer development and treatment
The major goal of this project is to decode the critical tumor suppressor functions and downstream components of a cancer-causing biochemical pathway that is amongst the most frequently mutated in human lung cancer.
Role: PI

5 R01 DK080425-07 (Shaw, PI) 07/01/2012-06/30/2016
NIH/NIDDK
LKB1-AMPK pathway regulation of glucose metabolism and metformin action in liver
The major goal of this project is to further dissect the role of AMPK and its related SIK family kinases in control of hepatic metabolism and therapeutic action of metformin in mouse models.
Role: PI

5 P01 CA120964-07 (Kwiatkowski, PI) 09/21/2012-07/31/2017
NIH/NCI
Molecular Pathogenesis of the Hamartoma Syndromes
The major goal of Project 2, LKB1/AMPK Signaling and Peutz-Jeghers Syndrome (L. Cantley, Project Leader, R. Shaw, Project Co-Leader), is to gain a detailed understanding of the LKB1-AMPK signaling network so that we can design therapeutic approaches to selectively attack tumors that emerge in patients with either germline or sporadic mutations in LKB1.
Role: Co-Investigator

W81XWH-13-1-0043 (Shaw, PI) 04/01/2013-03/31/2016
Department of Defense
Defining the role of autophagy kinase ULK1 signaling in therapeutic response of Tuberous Sclerosis Complex to mTOR inhibitors
The major goal of this project is to test how inhibition of ULK1 may change the normally cytostatic effect of rapalogs and other mTOR catalytic inhibitors in TSC-deficient cells into a cytotoxic effect.
Role: PI

Early Career (Shaw, PI) 09/15/2009-08/31/2015
Howard Hughes Medical Institute
Nutrient-Sensing Signaling Pathways Controlling Cancer and Diabetes
The major goal of this project is to elucidate mechanisms by which cells connect nutrient availability to cell growth and metabolism.
Role: PI

Research Grant (Evans/Shaw, PIs) 07/01/2013-06/30/2014
Samuel Waxman Cancer Research Foundation
Dietary stress-induced epigenetic signatures in cancer
The major goal of this project is to understand the consequences of environmental (high fat diet) and genetic (LKB+/-) stressors on specific tissue's propensity for cancer.
Role: Co-Investigator

COMPLETED

5 P01 CA120964-05 (Cantley, PI) 04/24/2007-03/31/2012
NIH/NCI
Molecular Pathogenesis of the Hamartoma Syndromes
The major goal of our project, LKB1/AMPK Signaling and Peutz-Jeghers Syndrome, is to further elucidate the signal transduction pathways that inhibit hamartomas formation in humans, focusing on the recently discovered role of the LKB1 tumor suppressor in regulating mTOR signaling via the TSC proteins.
Role: PI, Project 2

5 R01 DK080425-05 (Shaw, PI) 09/30/2007-06/30/2012
NIH/NIDDK
Role of LKB1 and AMPK in Metformin and TZD Control of Glucose Metabolism in Liver
The major goal of this project is to determine the role of LKB1 and AMPK in the control of hepatic glucose metabolism and in the therapeutic action of metformin and TZDs.
Role: PI

RSG-07-210-01-MGO (Shaw, PI) 07/01/2007-06/30/2011
American Cancer Society
The role of mTOR signaling in LKB1-dependent tumorigenesis
The major goal of this project is to further dissect how the LKB1 tumor suppressor inhibits the function of the pro-growth mTOR enzyme, as well as to perform preclinical trials on a mouse model of Peutz-Jeghers syndrome and a variety of human lung cancer cell lines to define the therapeutic potential of mTOR inhibitors against tumors with LKB1 mutations.
Role: PI

Research Grant (Evans / Shaw, co-PIs) 07/01/2010-06/30/2011
Samuel Waxman Cancer Research Foundation
Mechanisms of nutrient- and inflammation-induced intestinal cancers
The major goal of this project is to provide insights into the molecular mechanisms and the contributing role of inflammation in diet-induced intestinal cancer, and evaluate two new target and pathway specific therapeutic approaches for cancer treatment.
Role: Co-Investigator

Research Grant (Evans, PI) 07/01/2011-06/30/2012
Samuel Waxman Cancer Research Foundation
Mechanisms of nutrient- and inflammation-induced intestinal cancers
The major goal of this project is to provide insights into the molecular mechanisms and the contributing role of inflammation in diet-induced intestinal cancer, and evaluate a pathway-specific therapeutic approach for cancer treatment.
Role: Co-Investigator

Research Grant (Evans/Shaw, PI) 07/01/2012-06/30/2013
Samuel Waxman Cancer Research Foundation
Chronic Inflammation and Metabolic Dysregulation in Cancer
The major goal of this project is to provide insight into the molecular mechanisms and the contributing role of inflammation and bile acid homeostasis in diet-induced intestinal cancer, and evaluate a pathway-specific therapeutic approach for cancer treatment.
Role: Co-Investigator